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SCINTIGRAPHY FOR PULMONARY CAPILLARY PROTEIN LEAK

Annual Summary Report

Harvey J. Sugerman, M.D., James L. Tatum, M.D.
Jerry I. Hirsch, Pharm.D., and Alfred M. Strash, Ph.D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Pulmonary gamma scintigraphy is a rapid, non-invasive technique for measuring a pulmonary capillary protein leak in ARDS. In these studies, the method demonstrated that the acute anesthetized lung lymph fistula sheep model was associated with a significant pulmonary capillary protein leak. No leak was demonstrated in dogs following either large doses of Intralipid, sodium morrhuate or endotoxin. The protein leak following oleic acid could not be prevented with methylprednisolone (30 mg/kg), ibuprofen (12.5 mg/kg), MK-447		

44 mg/kg), or calcium gluconate (140 mg/kg). A significant and persistent leak followed the instillation of 0.1 hydrochloric acid into the trachea of dogs. This was not altered with pre-treatment with 30 mg/kg methylprednisolone or 12.5 mg/kg ibuprofen. Prostacyclin (PGI₂) infusion at 0.3 mcg/kg/min increased the rate of leak, as measured by the SI, by 50%. A significant SI was noted in pigs following the intravenous infusion of *Pseudomonas* (1×10^9 orgs/kg/min). Studies will be undertaken to investigate the ability of various agents (methylprednisolone, ibuprofen, PGI₂) to alter the SI following *Pseudomonas* infusions in the pig. Atelectasis was produced in 5 dogs by inflation of a balloon in the right main bronchus and repeat SI's were obtained. Atelectasis did not produce an increased SI. These studies will be compared to a group of dogs in which a right lobar pneumococcal pneumonia will be produced.

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SUMMARY

Computerized scintigraphy, employing the gamma camera, has been used in this contract to study the dynamics of the pulmonary capillary membrane leak of ^{99m}Tc -tagged human serum albumin (Tc-HSA). In preliminary canine studies, the severity of an oleic acid-induced albumin leak was proportional to the slope of lung:heart radioactivity ratio and was more sensitive than arterial blood gases or standard chest roentgenograms. We have called this rising ratio the "slope of injury" or "slope index" (SI). Our first studies were to compare this technique to the sheep lung lymph fistula endotoxin model. Following cannulation of the right thoracic caudal efferent lymph duct, we noted that these animals had evidence of a "slope of injury" prior to the administration of endotoxin. In order to determine whether the acute sheep model developed evidence of pulmonary injury, we compared sheep with thoracotomy, retraction of the lung and lymphatic cannulation to sheep that had undergone thoracotomy only. These data demonstrated a significantly higher "slope of injury" ($p < 0.01$) following thoractomy, lung retraction and lymph duct cannulation. A significantly increased SI was also noted in animals undergoing thoracotomy only. This suggests that the acute sheep lung lymph model is associated with acute pulmonary injury and, therefore, will make subsequent manipulation of the model difficult to interpret. In the first 1-1/2 years of this contract, we studied a number of agents in an attempt to prevent oleic acid-induced pulmonary microvascular injury. Following a series of five control dogs, five dogs each were studied with each of the following agents: methylprednisolone (30 mg/kg), ibuprofen (12 mg/kg), the superoxide radical scavenger, MK-447 (4 mg/kg), and, in three dogs, calcium gluconate (140 mg/kg). Each of these agents was given five minutes prior to administration of oleic acid (0.05 ml/kg). None of these agents was able to

alter the rise in lung: heart radioactivity ratio following oleic acid injury. In another study, we have administered 0.1 N hydrochloric acid, 2 ml/kg, into the trachea of dogs in the right lateral decubitus position and have found an acute and reproducible "slope of injury" similar to that seen with 0.05 ml/kg oleic acid. Neither pharmacologic doses of methylprednisolone (30 mg/kg) or ibuprofen (12.5 mg/kg) were able to alter the SI in 5 dogs each when given 5 minutes prior to instillation of 2 ml/kg 0.1 N HCl into the right mainstem bronchus. Infusion of prostacyclin (PGI_2), 0.3 mcg/kg/min, produced a 50% increase in the slope index. Methylprednisolone or ibuprofen failed to prevent the rise in pulmonary artery pressure or pulmonary vascular resistance or deterioration in PaO_2 following HCl instillation. PGI_2 , as expected, decreased systemic arterial pressure and preserved cardiac output following HCl. It is presumed that the increased rate of protein leak following the infusion of PGI_2 was due to preservation of flow to the injured pulmonary microvasculature.

A septic pig model is being developed for study of bacterially induced ARDS. Following the i.v. infusion of 1×10^9 Pseudomonas organisms at 1 ml/kg, a marked rise in SI was seen. Five control animals, infused with saline alone, had no rise in SI. We plan to measure thromboxane and 6-keto- $\text{PGF}_{2\alpha}$ (the stable metabolite of prostacyclin) as well as SI and extra-vascular lung water (EVLW) using the thermal-cardiogreen technique in pigs following i.v. pseudomonas. Later manipulations with prostaglandin blockers are planned.

A series of five dogs have undergone the production of right lobar atelectasis following obstruction of the right mainstem bronchus with inflation of a Carlen's tube balloon. This produced a shift of the heart to the right, necessitating redefining the scintigraphic areas of interest over the heart and right lung. Analysis of the lung:heart radioactivity ratios following atelectasis revealed a flat SI. Thus, atelectasis does not produce increased

pulmonary albumin flux. These studies will be compared to a group of dogs in whom a right lobar pneumococcal pneumonia will be produced by the instillation of pneumococci into the right mainstem bronchus. Presumably, a bacterial pneumonia will be associated with increased protein flux and a positive SI.

FOREWORD

In conducting the research described in this report, the investigators adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 78-23, Rev. 1978).

A. Problem

The acute respiratory distress syndrome (ARDS) is an ill-defined disorder with multiple etiologies which usually requires mechanical ventilation. Combat soldiers acquire this disorder from direct lung contusion, burn inhalation injury, inhalation of toxic substances as a result of chemical warfare, aspiration, multiple transfusions, as a complication of sepsis, etc. The National Heart, Lung and Blood Institute, Division of Lung Diseases, Task Force on Research in Respiratory Diseases estimated that 150,000 cases occur each year (1). Many of these are young, previously healthy persons. The overall mortality is impossible to assess, but must be quite high. Ninety-one percent of the 90 patients enrolled in the Extra-Corporeal Membrane Oxygenator study (ECMO), most of whom probably had ARDS, died (1). Data from the nine centers participating in the ECMO study showed that more than 75% of the 600 patients receiving inspired oxygen concentration (FiO_2) greater than 50% died (1). From 1973 to 1976, 119 patients were admitted to the Respiratory and Surgical Intensive Care Units of San Francisco General Hospital with a diagnosis of ARDS (7% of all their intensive care unit admissions) and of these, 53% died (1). Although the majority of these deaths were not solely due to respiratory failure, this probably affected their morbidity and mortality.

B. Background

The initial pathophysiologic event in ARDS is thought to be a leak in the pulmonary capillary membrane. This leads to an increase in pulmonary interstitial water and protein which is then removed by the pulmonary lymphatics. If the leak exceeds the lymphatic capacity, which can increase flow by a factor of 20, pulmonary interstitial edema occurs. When the interstitial compartment reaches a critical volume and pressure, the alveolar space abruptly fills. This causes an inhibition, or wash-out, of surfactant, which will

produce alveolar collapse and a reduced FRC. A ventilation-perfusion (V/Q) mismatch develops, with right-to-left pulmonary shunting and arterial hypoxemia.

There is a need for an accurate, sensitive, reproducible and noninvasive technique to measure the severity and duration of pulmonary capillary leakage in patients with ARDS. This will permit improvement in both the early diagnosis of this pathological condition as well as the objective evaluation of therapeutic interventions.

Attempts to document and quantitate the leakage of water and protein through the pulmonary capillary membrane have been fraught with frustration. Previous studies have been directed at attempts to measure pulmonary extravascular lung water (EVLW) using isotopic indicator dilution techniques. These methods, based on studies by Chinard and Enns (2) have been shown by Korngren et al. (3) and Marshall et al. (4) to be inaccurate, since they are flow dependent. A thermodilution-indocyanine green dye technique has also been applied to measure EVLW (5,6). This method does not appear to be as sensitive to changes in cardiac output as the isotopic indicator dilution techniques because of the much greater diffusability of the thermal indicator (7,8). The method provides a static estimate of extravascular lung water and would have to be repeated frequently to determine the dynamics of the leak. Brigham et al. (9) have developed a technique requiring the measurement of concentration-time curves for four radioactive agents (^{51}Cr -erythrocytes, ^{125}I -albumin, ^{14}C -urea, and ^3H -water) from which the extravascular lung water and the ^{14}C -urea permeability-surface area product is calculated. This technique would also provide a static estimate of extravascular lung water, seems cumbersome to use, and would also be affected by alterations in pulmonary vascular recruitment. Using a gamma probe technique, Gorin et al. (10) demonstrated a leak of $^{113\text{m}}$ Indium transferrin from the chest of sheep following

the intravenous injection of Pseudomonas aeruginosa bacteria. The intensity of radioactive counts correlated with the directly measured accumulation of this isotope in lung lymph. The position and aim of the gamma probe would have to be unaltered for the technique to be reproducible. It requires gamma well-counter analysis of serum samples which may limit widespread clinical utilization.

Gamma Scintillation Camera Technique: In studies from our laboratory, the computerized gamma camera was able to record and quantitate the pulmonary capillary membrane leak of 99m technetium-tagged human serum albumin (Tc-HSA) in dogs following intravenous oleic acid (11,12). This technique compares the change in radioactivity over the lung to that over the heart with the construction of a lung:heart radioactivity ratio.

Radioactive Tc-HSA distributes within the whole body blood pool after intravenous injection and remains essentially within the vascular compartment. Its distribution within the body can be imaged with the gamma camera. Using the computerized gamma camera, data are collected at one second intervals for 60 seconds and then at one-minute intervals for the duration of the study. During the initial pass of the radiopharmaceutical, it is possible to define the lungs and the heart anatomically for subsequent computer analysis and construction of lung:heart radioactivity ratios. This ratio remains constant unless a pulmonary microvascular membrane injury is present when a rising ratio is present. We have called this rising ratio the "slope index" (SI).

In previous canine oleic acid studies, we have found that the SI was proportional to the severity of injury and was more sensitive than either arterial blood gas analysis or standard chest roentgenograms (12). Using this method in the oleic acid model, it was found that the leak of Tc-HSA was much

greater than the leak of ^{99m}Tc -tagged RBC's (13), that PEEP did not alter the rate of pulmonary capillary protein leak (14), that altered pulmonary vascular recruitment did not produce a rising radioactivity ratio following hemodynamic equilibration (15) and that multiple doses of Tc-HSA were associated with reproducible SI's over six hours following oleic acid administration (16). The method is noninvasive and has been used clinically to determine the severity and duration of non-cardiogenic pulmonary edema (17).

C. Approach to the Problem

The animals were anesthetized, intubated and placed beneath a Pho-Gamma IV scintillation camera fitted with a low energy, parallel hole collimator. Data were collected on "floppy disc" using a DEC mobile gamma acquisition system and transferred to a DEC medical computer for the determinations of regions of interest. Data were collected at one frame per second for 60 seconds following the Tc-HSA and then at one frame per minute for the duration of the study. Lung:heart radioactivity ratios were performed on a Xerox Sigma-5 computer. SI's were calculated from 15 to 45 minutes following administration of Tc-HSA. Animals subsequently found to have a significant SI during the control period were presumed to have a primary pulmonary illness (i.e., viral pneumonia) and were deleted from the study group. Intravascular pressures were measured with a Brush-Gould, Model 2400, 4-channel recorder utilizing a Statham strain-gauge transducer. Cardiac outputs were measured with a Kim-Ray, Model 3500 E thermodilution cardiac output computer.

1. Sheep Studies

Computerized gamma scintigraphy has compared favorably to wet to dry lung weight ratios, alveolar epithelial membrane permeability, canine lymph flow, standard radiography and light microscopy for the measurement of pulmonary

microvascular permeability to albumin. The effects of altered pulmonary vascular recruitment and positive end-expiratory pressure on the scintigraphic lung:heart radioactivity have also been studied. Currently, measurement of volume and protein concentration of lymph from the right caudal efferent lymph duct of sheep is the accepted model for the study of pulmonary permeability edema (18). It is, therefore, necessary to compare our scintigraphic technique to this model. We believed that an increased pulmonary microvascular permeability, associated with an increased lung lymph flow and protein concentration would be accurately detected by alterations in the scintigraphic "slope index." In addition, it should be possible to quantitate the amount of technetium-tagged albumin appearing in the lung lymph using a "well" counter. Several attempts were made to scan awake sheep suspended beneath the gamma camera in the upright position. However, the sheep could not be kept immobilized nor were posterior images adequate for accurate lung:heart radioactivity ratios. Therefore, the sheep were anesthetized, intubated, and placed in the supine position for anterior imaging and ventilated at a tidal volume of 20 ml/kg with 50% O₂ and 5 cm PEEP.

Initially, the sheep were subjected to thoractomy and right caudal thoracic lymph duct cannulation shortly before scanning and administration of endotoxin, the first perturbation we planned to investigate. However, sheep had very high SI's during the "control" period (see Results). These data led us to hypothesize that the "acute" lung lymph sheep model was associated with significant pulmonary injury, which would make it difficult to interpret a perturbation such as endotoxin. We, therefore, elected to scan five anesthetized sheep for one hour following 10 mCi Tc-HSA, perform a thoracotomy and right caudal thoracic lymphatic duct cannulation (which is associated with retraction of the right lung for 30 to 60 minutes) close the chest and re-expand the lung, inject another 10 mCi Tc-HSA and scan again for one hour. These results were

compared to two groups of five sheep each who were either scanned before and after thoracotomy only, without lung retraction or lymph duct cannulation or sheep who underwent anesthesia alone. We are currently studying sheep 48 to 72 hours following lymph duct cannulation to determine if the pulmonary injury will resolve with time.

2. Canine Studies

Dogs weighing approximately 20 kg, were anesthetized with 30 mg/kg sodium pentobarbital, intubated, and ventilated at a tidal volume of 20 ml/kg with 50% O₂ and 5 cm H₂O positive end-expiratory pressure (PEEP).

a. Oleic Acid Injury: This model was chosen because of our extensive previous experience with it as well as the possible relationship of ARDS to free fatty acids in pancreatitis and the traumatic pulmonary fat embolism syndrome. We attempted to block the rising SI seen with oleic acid pulmonary microvascular injury with the following agents administered to five dogs each five minutes prior to 0.05 ml/kg oleic acid: methylprednisolone (30 ml/kg), the non-steroidal anti-inflammatory prostaglandin blocker, ibuprofen (12 mg/kg), the superoxide radical scavenger, MK-447 (4 mg/kg), and calcium gluconate (140 mg/kg). If any agent proved effective, we planned to administer it at various time intervals following oleic acid. However, no agent was found to reduce the rising SI after oleic acid (19).

b. Sodium Morrhuate (NaMor): A few patients with bleeding esophageal varices treated by endoscopic variceal sclerosis with sodium morrhuate have developed ARDS. NaMor is a 5% mixture of several fatty acid salts (8% palmitate, 12% palmitic oleate, 1% stearate, 2% oleate, 28% linoleate, 15% arachidate, 8% arachidonate, and 26% other). ARDS in these patients could either be secondary to aspiration of gastric contents and blood, not unusual in a cirrhotic patient with reduced mental function, or fatty acid injury.

Five dogs, weighing approximately 20 kg, were given 5 ml and 2 dogs 20 ml of NaMor intravenously one hour after 10 mCi Tc-HSA. Second and third doses of 10 mCi Tc-HSA were given one and three hours, respectively, after NaMor.

c. Intralipid: This solution of neutral fat has been used in association with total parenteral nutrition and has been incriminated as a possible cause of ARDS in critically ill patients (20). Hyperlipidemia has also been implicated in ARDS associated with acute pancreatitis (21). To investigate this possibility in our dog model, four dogs were given 500 ml of 20% intralipid over 15 minutes one hour after 10 mCi Tc-HSA. This equals 100 grams of neutral fat, a very large dose. Pulmonary arterial pressure (PAP), pulmonary capillary wedge pressures (PCWP), and systemic arterial pressure (SAP) were monitored throughout the study. Cardiac outputs were measured every 15 minutes. The animals were scanned continuously beneath the gamma camera. A second and third dose of 10 mCi Tc-HSA were given two and four hours after intralipid, respectively. Two dogs received 500 ml of 20% intralipid which was followed in 30 minutes by 3000 units of sodium heparin in order to activate lipoprotein lipase, release free fatty acids, and possibly produce ARDS analogous to the intravenous administration of oleic acid. Plasma samples were obtained before and after intralipid and then 1 and 2 hours following heparin for measurement of plasma free fatty acids. Scintigraphic SI's were obtained before, 1 hour after intralipid, and 1 hour after heparin

d. HCl: Aspiration of gastric contents is a known complication of traumatized individuals as well as a well-recognized postoperative complication. In this study, eight dogs were anesthetized with sodium pentobarbital, intubated, ventilated at a tidal volume of 20 ml/kg, and placed beneath a computerized gamma camera. Two ml/kg 0.1 N HCl were instilled into the right mainstem bronchus of dogs placed in the right lateral decubitus position for

30 minutes. Second and third doses of 10 mCi Tc-HSA were given 1/2 hour and 1-1/2 hours after HCl, respectively. Five animals received HCl instillation alone and five animals each were pretreated with methylprednisolone (30 mg/kg), ibuprofen (12.5 mg/kg) 5 minutes prior to acid instillation or prostacyclin (PGI_2) as a continuous infusion (0.3 mcg/kg/min) begun 30 minutes prior to HCl instillation and continued for 3 hours after administration of acid.

e. Atelectasis versus Bacterial Pneumonia: We were concerned that an atelectatic lung would be associated with a rising SI and increased albumin flux. Therefore, right lobar atelectasis was produced by balloon inflation in the right mainstem bronchus using a Carlen's tube. This produced an acute shift of the heart to the right into the scintigraphic pulmonary area of interest. It was, therefore, necessary to redefine the scintigraphic areas of interest over the heart and right lung following the production of atelectasis. A repeat dose of 10 mCi Tc-HSA was then administered and the SI constructed. The animals were sacrificed at the end of the study and the chest opened to ensure that the right lung had become atelectatic. These animals will be compared in the future to a group of animals who undergo the production of a right lobar pneumococcal pneumonia following the instillation of 2.5 ml of 6×10^7 CFU pneumococci into the right mainstem bronchus. Animals will be scanned prior to bacterial instillation and the scintigraphic study repeated 48 hours after the production of pneumococcal pneumonia. The SI's will be compared to standard chest radiographs and wet to dry lung weight ratios.

3. Porcine Studies

It is not surprising that injuries produced by intravenous oleic acid or transbronchial instillation of HCl were not prevented with corticosteroids or prostaglandin blockers, since these appear to be direct, non-mediated injuries

to the pulmonary alveoli and microvasculature. It is quite probable that ARDS secondary to sepsis is, in part, mediated by the release of prostaglandins (22) and complement (23) and is associated with both leukocyte (24) and platelet (25) aggregation in the pulmonary microvasculature. Septicemia is also one of the leading causes for fulminant ARDS and might be, theoretically, ameliorated with pharmacologic agents. We have, therefore, begun studies using a porcine pseudomonas septicemia model which has been shown to be associated with a fulminant ARDS (26). We plan to compare the SI, EVLW (measured by the thermal-cardiogreen technique), thromboxane and 6-keto-PGF_{2α} production, pulmonary and systemic hemodynamics in pigs given a saline infusion to pigs given an infusion of 1×10^9 pseudomonas (PAL strain) organisms at a rate of 1 ml/kg/min.

D. Results

1. Sheep

a. Five sheep were found to have high SI's during the "control" period following thoracotomy and lymph duct cannulation. The effect of endotoxin administration in these animals could not be interpreted.

b. Eight sheep were studied prior to and following thoracotomy without lung retraction or lymphatic duct cannulation. Three animals had a high SI during the control period and were deleted from the study. The SI's in the remaining five sheep were normal during the control period. In two sheep the SI was normal and in three the SI was $> 1 \times 10^{-3}$ U/min following thor cotomy. This suggests that thoracotomy with lung collapse and re-expansion alone may produce an increased albumin flux associated with an elevated SI. Re-expansion following pneumothorax has been shown to be associated with an increased lung water (27).

c. Seven sheep were studied prior to and following thoracotomy, lung retraction, and lymphatic duct cannulation. Two animals had a high SI during the control period and were deleted from the study. The mean control SI in the remaining five sheep was $0.6 \pm 0.6 \times 10^{-3}$ U/min. Following thoracotomy, lung retraction and lymphatic duct cannulation, the SI rose significantly ($p < 0.01$) to $1.3 \pm 0.6 \times 10^{-3}$ U/min. The acute lymphatic duct cannulation model, therefore, appears to have a significant injury to the lung according to the scintigraphic pulmonary capillary protein leak technique. Similar results have been noted by other investigators (28).

2. Dogs

a. Oleic Acid Injury: Of 28 dogs studied, 5 dogs had an SI greater than 0.6×10^{-3} U/min during the control period and were deleted from the study. The following data were obtained (35):

<u>Study Group</u>	<u>SI ($\times 10^{-3}$ U/min)</u>
Control oleic acid (OA) 0.05 ml/kg	2.9 ± 1.0
Methylprednisolone (30 mg/kg) + OA	3.8 ± 1.9
Ibuprofen (12 mg/kg) + OA	3.8 ± 0.8
MK 447 (4 mg/kg) + OA	2.9 ± 0.3
Ca ⁺⁺ Gluconate (140 mg/kg) + OA	3.0 ± 0.4

Therefore, none of the agents tested were able to prevent scintigraphic evidence of an oleic acid pulmonary microvascular injury.

b. Sodium Morrhuate (NaMor): Neither 5 ml nor 20 ml of NaMor produced a significant rise in SI over five hours of study following multiple doses of Tc-HSA. A slight, but transient, rise in PAP was seen following NaMor. There was no change in PCWP. This neutral salt preparation of fatty acids does not produce pulmonary microvascular injury seen following the free fatty acid, oleic acid.

c. Intralipid: A marked increase in PAP (from 17 ± 4 to 32 ± 6 mmHg) was seen following 500 ml of 20% intralipid with a modest rise in PCWP (from 8 ± 3 to 16 ± 2) in each of the 4 dogs. The serum became grossly lipemic. Nevertheless, no rise in SI was seen over five hours in any animal. One must question any relationship of increased serum neutral fat to ARDS. Similar results have also been noted in a sheep lung lymph cannulation study (24). We are currently attempting to release free fatty acids following intralipid infusions with either heparin or lipoprotein lipase. Our results are too preliminary to report at this time.

d. HCl Studies: Instillation of 2 ml/kg 0.1N HCl into the right mainstem bronchus of dogs produced an acute increase in pulmonary vascular resistance, from 164 ± 58 to 281 ± 67 dynes-sec-cm⁻⁵, and SI, from 0.4 ± 0.4 to 2.0 ± 1.0 , within 30 minutes which persisted for the next 90 minutes. These changes were not ameliorated with pretreatment, 5 minutes prior to acid instillation, with either 30 mg/kg i.v. methylprednisolone or 12.5 mg/kg i.v. ibuprofen. Infusion of PGI₂ at 0.3 mcg/kg/min produced a significant increase in both cardiac output and slope index following acid instillation over control values, but did not alter pulmonary artery pressure. The increased albumin flux as determined by the increased SI following PGI₂ was presumably due to increased flow to the injured pulmonary microvasculature.

3. Porcine Studies

Five pigs, weighing 30 to 40 kg, underwent anesthesia with pentobarbital (30 mg/kg), intubation, insertion of arterial and Swan-Ganz catheters. Control scintigraphic studies were obtained over 3 hours and the SI remained 0.4×10^{-3} U/min following 3 injections of Tc-HSA. One pig was given an i.v. infusion of 1×10^9 pseudomonas organisms at a rate of 1 ml/kg/min. This was associated with a marked and sustained increase in pulmonary artery pressure, fall in

cardiac output, and marked increase in SI to 2×10^{-3} U/min. Further studies are planned which will include measurement of thermal-cardiogreen EVLW, thromboxane, and 6-keto-PGF₂ α production.

E. Conclusions

The sheep studies showed a significant rise in SI following thoracotomy, lung retraction and lymphatic duct cannulation. Furthermore, thoracotomy alone produced a rise in SI in several animals. Therefore, the acute lung lymph cannulation model appears to be associated with a significant pulmonary microvascular injury. Additional studies will also be necessary with sheep 72 hours following the lymph cannulation procedure to determine if injury persists in the chronic sheep lymph model.

The dog studies showed that none of the agents tested (methylprednisolone, ibuprofen, MK-447 or calcium gluconate) were able to alter the scintigraphic evidence of oleic acid induced pulmonary microvascular injury when given prior to the administration of oleic acid. Several studies have suggested that methylprednisolone will prevent oleic acid injury (29,30); whereas, other studies have found no effect on lung water with this medication (31). The data from our study are quite clear that pharmacologic doses of methylprednisolone had no effect on the pulmonary capillary leak of albumin.

Similar negative results were seen following pretreatment of dogs following acid instillation with either pharmacologic doses of methylprednisolone or ibuprofen. Infusion of prostacyclin (PGI₂) at 0.3 mcg/kg/min produced an increase in SI compared to control dogs as well as an increase in cardiac output. Presumably the increased albumen flux noted with PGI₂ was secondary to an increased flow across the injured pulmonary microvasculature.

Studies in dogs following the production of unilateral lobar atelectasis revealed no evidence of abnormal albumin flux in the atelectatic lobe. These data will be compared in the future to animals in whom lobar pneumonia is produced with the bronchial instillation of pneumococci. These animals will presumably have increased albumin flux in the pneumonic lobe. The technique may be of value clinically in differentiating between an atelectatic versus a pneumonic radiographic infiltrate.

The porcine model of ARDS secondary to the intravenous infusion of *Pseudomonas* shows promise for the study of prostaglandin blockade in modifying the rate of albumin flux. Control studies have been completed. Studies following the infusion of *Pseudomonas* have begun.

The studies of the effect of sodium morrhuate (NaMor) in dogs demonstrated no scintigraphic evidence of a pulmonary capillary protein leak. No change in pulmonary artery pressures (PAP) were noted in these dogs following NaMor. A study of this agent using the lung lymph technique in sheep performed in another laboratory in our institution demonstrated a marked rise in PAP and an increase in lymph flow with a fall in lymph protein concentration. This agent, therefore, does not appear to cause permeability pulmonary edema in either the sheep or dog. The ARDS seen in patients following sclerotherapy of esophageal varices is, therefore, probably related to aspiration of gastric contents or blood or, perhaps, secondary to a marked increase in PAP.

Massive infusions of intralipid produced grossly lipemic serum and pulmonary artery hypertension but did not cause scintigraphic evidence of a pulmonary capillary protein leak. Infusions of heparin to activate lipoprotein lipase, or infusion of this enzyme itself, might release free fatty acids which would then produce pulmonary microvascular injury. These studies are too preliminary to report.

F. Recommendations

Pulmonary contusion and the acute respiratory distress syndrome were major complications of the Korean conflict, where it was termed the "Traumatic Wet Lung Syndrome," and Viet Nam, where it was called "Da Nang Lung" (32-35). A major cause of death following trauma is sepsis which leads to multi-system organ failure. In a 1977 study by Eiseman et al. (36), 42 patients were found with multiple system organ failure of whom 29 were septic and 19 died. Average hospital costs, excluding physician's fees, were conservatively estimated at \$21,000. Fry et al. (37) found that sepsis was the most common cause of multiple system organ failure and this was the most common fatal expression of severe sepsis. Of 553 consecutive emergency surgical patients, 55 died post-operatively. Of these, infection was the cause of death in 32. Thirty-four of 123 septic patients had multiple system organ failure. The lung is the most common organ injured in the septic patient.

There is no currently accepted objective measurement of a pulmonary capillary protein leak that can be used clinically. The thermal-cardiogreen method will purportedly measure the amount of lung water that has already leaked (5-8), but does not determine if an active protein leak is occurring.

The proposed method of computerized pulmonary gamma scintigraphy is conceptually simple, noninvasive, reproducible and should permit the objective evaluation of the presence and duration of ARDS, and its response to therapeutic interventions. Should a treatment be found to be efficacious, the severity of ARDS should be reduced. This would include a reduced need for prolonged mechanical ventilation as well as a decrease in the attendant morbidity associated with the leakage of proteinaceous fluid with its associated bacterial pneumonia and pulmonary fibrosis. This should improve the resuscitation of the severely injured combat soldier so that he can be returned sooner to active duty or be more rapidly rehabilitated.

The scintigraphic results suggest that the acute sheep lung lymph model is an unreliable model since it is associated with severe lung injury prior to any perturbation. Additional studies are necessary to evaluate a chronic sheep model.

A number of sheep and dogs were found to have rising lung:heart radioactivity ratios during the "control" period. These animals have been excluded from further analysis. However, the cause for this finding must be found. Possible etiologies include an incomplete binding of the technetium to the human serum albumin. Unfortunately, chromatographic analyses of the Tc-HSA were not performed in those studies. Subsequently, all preparations of Tc-HSA have been checked and found to have more than 98% binding. It is probable that these animals had a primary pulmonary condition such as a viral pneumonia (i.e., distemper). It could be ideal if lung:heart radioactivity ratios could be checked during the "control period" and, if a "slope of injury" is found, the study be aborted. This is technically very difficult at the present time since the "floppy discs" from the mobile gamma acquisition unit need to be taken to the clinical nuclear medium computer in another building, nine floors away, for analysis. Construction of a dual probe system should provide "on-line" data analysis using a microcomputer system. This would expedite data analysis as well as prevent long, expensive experiments in animals with concurrent disease.

No agents yet studied were able to reduce the increased albumin flux in animals given i.v. oleic acid or transbronchial instillation of HCl. These results are not surprising since both oleic acid and HCl probably produce a direct microvascular injury and do not act through the release of noxious intermediates. It is more likely that the injury following bacteremia is produced by the release of intermediates such as prostaglandins, complement

activation, and super-oxide radicals. The *Pseudomonas* porcine model holds promise for studying various blocking agents in modifying the rate of albumin flux in sepsis.

Massive doses of intralipid were not associated with scintigraphic evidence of pulmonary capillary protein leak. Because of the relationship of pancreatitis and pulmonary fat embolism to hyperlipidemia and ARDS, one must question whether free fatty acids must be released from the neutral fat before an injury can be seen. Infusions of intralipid will be combined with infusions of heparin, in order to activate lipoprotein lipase or combined with the intravenous administration of lipase. If scintigraphic evidence of a protein leak is seen, appropriate controls will be required.

In conclusion, computerized pulmonary gamma scintigraphy represents a rapid, noninvasive method for detection and assessment of the severity of a pulmonary capillary protein leak in experimental animals as well as the response to a therapeutic intervention. This should improve our understanding of the pathophysiology, as well as therapy, in combat soldiers who develop ARDS.

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Abstracts and Presentations

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1. Sugerman, H.J., Blocher, C.R., Hirsch, J.I., Tatum, J.L., and Strash, A.M.: Failure of methylprednisolone, ibuprofen and MK-447 to alter scintigraphic measurement of oleic acid permeability edema. *Circ. Shock* 9:197(A), 1982. Presented at Shock Society, Smuggler's Notch, Vermont, June, 1982.
2. Sugerman, H.J., Blocher, C.R., Hirsch, J.I., Tatum, J.L., and Strash, A.M.: Failure of methylprednisolone, ibuprofen and MK-447 to alter scintigraphic measurement of oleic acid permeability edema. *Circ. Shock* 9:197(A), 1982. Presented at Association Academic Surgery, San Diego, California, 1982.

Articles

1. Sugerman, H.J., Blocher, C.R., Hirsch, J.I., et al.: Failure of various agents to decrease oleic acid pulmonary albumin leak. *J. Surg. Res.* 34:456, 1983.